Stapfia 14 127 – 134 20. Mai 1985

# DEVIATING BASIC GENOME SIZE IN A HEXAPLOID POPULATION OF SCILLA BIFOLIA AGG. IN THE VALLEY KREUTTAL (WEINVIERTEL; LOWER AUSTRIA)

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Abstract.

Deviating basic genome size in a hexaploid population of Scilla bifolia agg. in the valley Kreuttal (Weinviertel, Lower Austria). Key words: Scilla, Liliaceae; polyploidy, genome size.

DNA content determinations in a hitherto unique hexaploid population (2n = 54) of Scilla bifolia agg. in the valley Kreuttal, Weinviertel, Lower Austria, revealed a basic DNA content of 1 $C_x$ = 5,01 pg (1 C = 15,03 pg), which is significantly less than the values of about 1 $C_x$ = 5,7 pg which are typical for the close allies Scilla bifolia subsp. danubialis (2n = 18, 2x), Scilla drunensis (2n = 36, 4x), or Scilla buekkensis (2n = 36 - 54, 4x - 6x). New measurements in S. drunensis from northern Lower Austria, and from the Vitoscha mountains near Sofia, Bulgaria, as well as reassessments of previous reports in S. bifolia subsp. danubialis, S. drunensis and S. buekkensis confirmed the previously reported basic genome size of about 1 $C_x$ = 5,7 pg. As to now, 1 $C_x$ = 5,01 pg is the lowest value recorded in Scilla sect. Scilla. The findings on the Kreuttal - population demand a search for other such populations and for possible ancestors of lower degree of polyploidy, until the biological and taxonomic significance of the present data can be reasonably assessed.

Zusammenfassung.

Abweichende Grund - Genomgröße in einer hexaploiden Population von Scilla bifolia agg. im Kreuttal (Weinviertel, Niederösterreich).

DNA - Mengenbestimmungen an einer bisher einzigartigen hexaploiden Population von Scilla

bifolia agg. im Kreuttal, Weinviertel, Niederösterreich, ergaben eine Grund - Genomgröße von 1 $C_x$ = 5,01 pg (1 C = 15,03 pg). Dies ist signifikant weniger als die Werte von etwa 1 $C_x$ = 5,7 pg, die für die nahen Verwandten Scilla bifolia subsp. danubialis (2n = 18, 2x), Scilla drunensis (2n = 36, 4x), oder Scilla buekkensis (2n = 36 - 54, 4x - 6x) typisch sind. Neue Messungen an S. drunensis aus dem nördlichen Niederösterreich und von den Vitoscha - Bergen bei Sofia, Bulgarien, sowie Überprüfungen früherer Befunde an S. bifolia subsp. danubialis, S. drunensis und S. buekkensis bestätigten die bereits früher publizierte Grundgenomgröße von etwa 1 $C_x$ = 5,7 pg. Gegenwärtig stellt 1 $C_x$ = 5,01 pg den niedrigsten bei Scilla sect. Scilla gefundenen Wert dar. Die Befunde über die Kreuttal - Population machen die Suche nach weiteren solchen Populationen und nach möglichen Ahnen mit niedrigerem Polyploidiegrad erforderlich, ehe die biologische und taxonomische Bedeutung der vorliegenden Daten vernünftig eingeschätzt werden kann.

## Introduction.

There exist only a few dispersed Lower Austrian populations of Scilla bifolia agg. north of the Danubian valley, which attracted attention not earlier than recently when Greilhuber and Karrer communicated the occurence of 2n = 54, 6x, for plants collected in the valley Kreuttal (Fig. 1,2). In the meantime, the remaining three populations, at Goggendorf, at Wischathal, and at Sonnberg near Hollabrunn, were identified, not quite expectedly, as S. drunensis, and the chromosome number 2n = 36, 4x, was stated (Speta 1983). As is well known since the studies of Speta (1973), the Lower Austrian part of the Danubian valley below Ybbs/Donau is populated by Scilla vindobonensis, 2n = 18, 2x, with the only exceptional disjunct population of Scilla bifolia subsp. danubialis, 2n = 18, 2x, at Stopfenreuth (Greilhuber and Speta 1977). The Kreuttal - plants are habitually, and in regard of the basic karyotype morphology, similar to S. bifolia subsp. danubialis or S. drunensis, and have brown seeds (an essential group character) like these taxa too. However, we report here that these plants have significantly less DNA per basic genome than S. bifolia, S. drunensis, or the tetra - hexaploid S. buekkensis. During the course of this study, for comparison new DNA determinations have also been performed in S. drunensis from Goggendorf, Lower Austria, and from the Vitoscha mountains near Sofia, Bulgaria. Furthermore, previously published values in S. bifolia subsp.

danubialis, S. drunensis, and S. buekkensis were reproduced in this study. These data corroborate a fairly stable and consistently higher basic DNA value in these taxa than we find in the Kreuttal - population.

## Material and methods.

Chromosome counts were performed with colchicinated root tip and ovule material. DNA contents were determined by Feulgen two - wavelength cytophotometry on a Leitz MPV II cytophotometer. Root tips, and bisected ovaries of flowering plants were fixed together with Allium cepa cv. 'Centennial' root tips as an internal standard in methanol - acetic acid (3 : 1) overnight at + 4°C, stored in 96% ethanol in the deep - freezer, and processed a few days later. Ovule material was scrupulously teased into pieces as small as possible with needles prior to the first wash before hydrolysis to avoid incomplete penetration of reagents due to epidermal cuticles. The classical Feulgen - reaction was performed in Eppendorf centrifuge tubes, because numerous centrifugations were required. The 50 min. 5N HCl hydrolysis at 20°C was applied. Air dried squash preparations were measured within 1 or 2 days. Immersion oil was used as mountant medium without a cover slip. From each slide the relative Scilla value, $\bar{q} \pm s_q$, was calculated, and the weighted means and standard deviations of all slides of a provenance, $\bar{q}_w \pm s_{q_w}$, formed (see Sachs 1978, pp. 63, 64, 77). Relative values were transformed to absolute ones taking 1 C = 16,75 pg for Allium cepa (Van't Hof 1965, Bennett and Smith 1976). Statistical tests were performed as indicated in the text (see Sachs 1978, pp. 209 - 216, 386 - 395). The species and provenances are as follows:

1. Scilla bifolia agg. Lower Austria, the valley Kreuttal, between Hetzmannsdorf and Unterolberndorf, 205 m to 225 m alt., flore mapping field square 7564/4. Plant material was collected at three points (see Table 1), in the western, the central and the eastern part of the valley: (a) long. 16°26'16" E, lat. 48° 26' 26,4" N, (b) long. 16° 27' 12" E, lat. 48° 26' 41,3"N (c) long. 16° 28' 00" E, lat. 48° 26' 36,4" N.
2. Scilla bifolia L. subsp. danubialis Speta. Lower Austria, water meadows along the Danube near Stopfenreuth, 144 m alt.
3. Scilla drunensis (Speta) Speta. Lower Austria, Goggendorf, hill "Kasperlberg", approx. 335m alt., leg. F. Speta.

4. Scilla drunensis (Speta) Speta. Upper Austria, Pucking near Traun, near the cottage farm "Oberreiter", 285 m alt.
5. Scilla drunensis (Speta) Speta. Bulgaria, Vitoscha mountains, near Sofia, approx. 1 200m.
6. Scilla buekkensis Speta. Hungary, Bükk mountains, 1,5 km NW Répàshuta, leg. F. Speta 23.3.1974, type material !

Herbarium specimens are preserved in the herbarium of F. Speta (Sp), Linz. Specimens of provenance (1) are also preserved in the herbarium of the Institute of Botany, University of Vienna (WU).

## Results and discussion.

The data of the present investigation are indicated in Tables 1 and 2, and present evidence that the basic DNA content 1 $C_x$ is significantly lower in the hexaploid Kreuttal population than in the diploid Scilla bifolia, the tetraploid S. drunensis, or the tetra- to hexaploid S. buekkensis. Analysis of variance shows that the basic DNA contents in the provenances 2 to 6 of S. bifolia, S. drunensis, and S. buekkensis are not significantly different from each other ($F = 2{,}14 < F_{4,217;0,05} = 2{,}42$), while inclusion of the Kreuttal plants indicates a highly significant difference ($F = 12{,}04 > F_{5,369;0,01} = 3{,}07$). The Scheffé - test indicates a higly significant difference of the Kreuttal mean and the mean of the other values ($S = 41{,}625 > S_{0,01} = 3{,}915$). Within the Kreuttal - population, analysis of variance indicates that some values may be different from each other ($F = 2{,}39 > F_{9,144;0,05} = 1{,}95$), but the "modified LSD (lowest significant difference) - test" indicates homogeneity of these values. One should also note that the values obtained from root tips are not different from those obtained from ovules ($\hat{t} = 0{,}253 < t_{151;0,05} = 1{,}976$) and that the means of the three Kreuttal subpopulations, (a), (b), and (c), are not significantly different from each other ($\hat{F} = 0{,}17 < F_{2,150;0,05} = 3{,}06$). Therefore, there is every reason to assume that the significant part of the variation within the Kreuttal plants is methodical and not genuine. There is also no evidence that the lower 1 C value in this population is a methodical artifact due to glare as a result of a presumably higher chromatin compaction in the hexaploids, since no such effects are observed, for instance, in the 2x- 4x- 6x-

Table 1. DNA measurements in plants of *Scilla bifolia* agg. from three subpopulations (a – c) of the valley Kreuttal (Weinviertel, Lower Austria).

| Subpopulation | Plant no. | Chromosome number | | N | 1 C (pg), $\bar{q}_w \pm s_{q_w}$ |
|---|---|---|---|---|---|
| a | 1 | 2n = 54 | | 10 | 15,26 ± 0,82 |
| | 2 | 2n = 54 | | 10 | 14,85 ± 0,67 |
| | 3 | 2n = 54 | | 10 | 14,87 ± 0,63 |
| | 4 | | | 9 | 15,37 ± 0,81 |
| | | | sum of a: | 39 | 15,08 ± 0,74 |
| b | 5 | | | 16 | 15,52 ± 0,68 |
| | 6 | | | 10 | 14,88 ± 0,67 |
| | 7 | | | 10 | 14,78 ± 0,36 |
| | 8 | | | 10 | 14,48 ± 0,63 |
| | | | sum of b: | 46 | 14,99 ± 0,72 |
| c | 9 | 2n = 54 | | 10 | 15,10 ± 0,76 |
| | 10 + 11 | 2n = 54 | | 58 | 15,01 ± 0,68* |
| | | | sum of c: | 68 | 15,02 ± 0,69 |
| | | | sum of 1-9: | 95 | 15,04 ± 0,73 |
| | | | sum of a-c: | 153 | 15,03 ± 0,71 |

*Values obtained from root tips; in the other plants the values were obtained from ovules.

Table 2. DNA measurements in provenances (as listed under materials and methods) of the *Scilla bifolia* alliance.

| Species | Provenance | Chromosome number | 1C (pg), $\bar{q}_w \pm s_{q_w}$ | N | $1C_x$ (pg) |
|---|---|---|---|---|---|
| *Scilla bifolia* agg. | 1 | 2n = 54, 6x | 15,03 ± 0,71 | 153 | 5,01 |
| *Scilla bifolia* subsp. *danubialis* | 2 | 2n = 18, 2x | 5,80 ± 0,26 | 50 | 5,80 |
| *S. drunensis* | 3 | 2n = 36, 4x | 11,22 ± 0,50 | 55 | 5,61 |
| *S. drunensis* | 4 | 2n = 36, 4x | 11,37 ± 0,53 | 56 | 5,69 |
| *S. drunensis* | 5 | 2n = 36, 4x | 11,62 ± 0,45 | 11 | 5,81 |
| *S. buekkensis* | 6 | 2n = 36, 4x | 11,55 ± 0,59 | 50 | 5,78 |

polyploid series of S. subnivalis, S. reuteri, and S. pneumonanthe (Greilhuber et al. 1981, see also Seal 1983). The main problem arising from the present data is the phylogenetic position of the Kreuttal population. Is it a hexaploid derivative of the geographically neighbouring allies S. bifolia or S. drunensis - or of a common ancestor of these - with a lower DNA

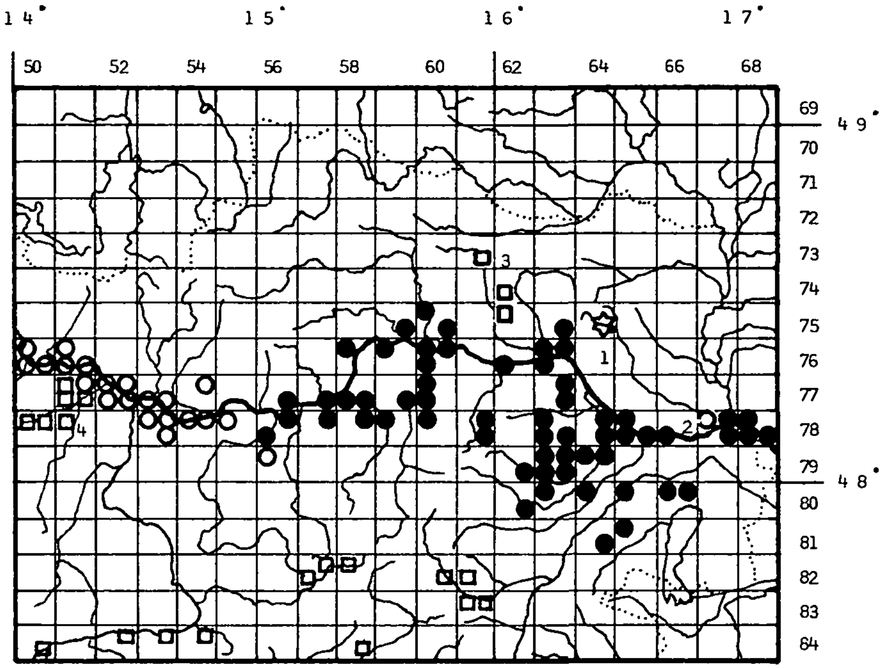


Fig. 1: The Austrian provenances of Scilla bifolia agg. measured (1 - 4) in the context of species distribution. Empty circles: S. bifolia subsp. danubialis (2n = 18); empty squares: S. drunensis (2n = 36); asterisk: S. bifolia agg. (2n = 54), Kreuttal; black circles: S. vindobonensis (2n = 18). Distributional map according to Speta (1983). The S. vindobonensis locality, field square 6375/4, is our addition: Rohrwald near Stockerau, (a) long. $16^{o}18'$ 24,4" E, lat. $48^{o}24'$ 38,4" N, (b) long. $16^{o}$ 18' 42,6" E, lat. $48^{o}$ 24' 48,0" N; chromosomal identification of fruiting plants.

content, or are these plants more directly phylogenetically related to the morphologically somewhat distinct S. laxa, known as tetra- and hexaploids, with a DNA content of about 1 $C_x$ = 5,2 pg? The published DNA amount for S. laxa (4x) from Talmaciu at Sibiu, Rumania, (Greilhuber 1979), given here as $\bar{q}_w \pm s_{q_w}$ in pg DNA, is 1 C = 10,43 $\pm$ 0,54, which amounts to a significant difference to the Kreuttal genome ($t = 5{,}158 > t_{201;0,001} = 3{,}340$). New unpublished measurements from other provenances of S. laxa rather reinforce this significant difference. Similar low DNA contents of about 5,3 pg or 5,4 pg (1 $C_x$) are found in the black seeded taxa S. subnivalis, S. reuteri, S. pneumonanthe, S. xanthandra and S. longistylosa, which form a monophyletic group together with S. nivalis and Scilla ser. Chionodoxa according to the present view of phylogenetic relationships in Scilla (Greilhuber, in press; Greilhuber 1979, Greilhuber et al. 1981).

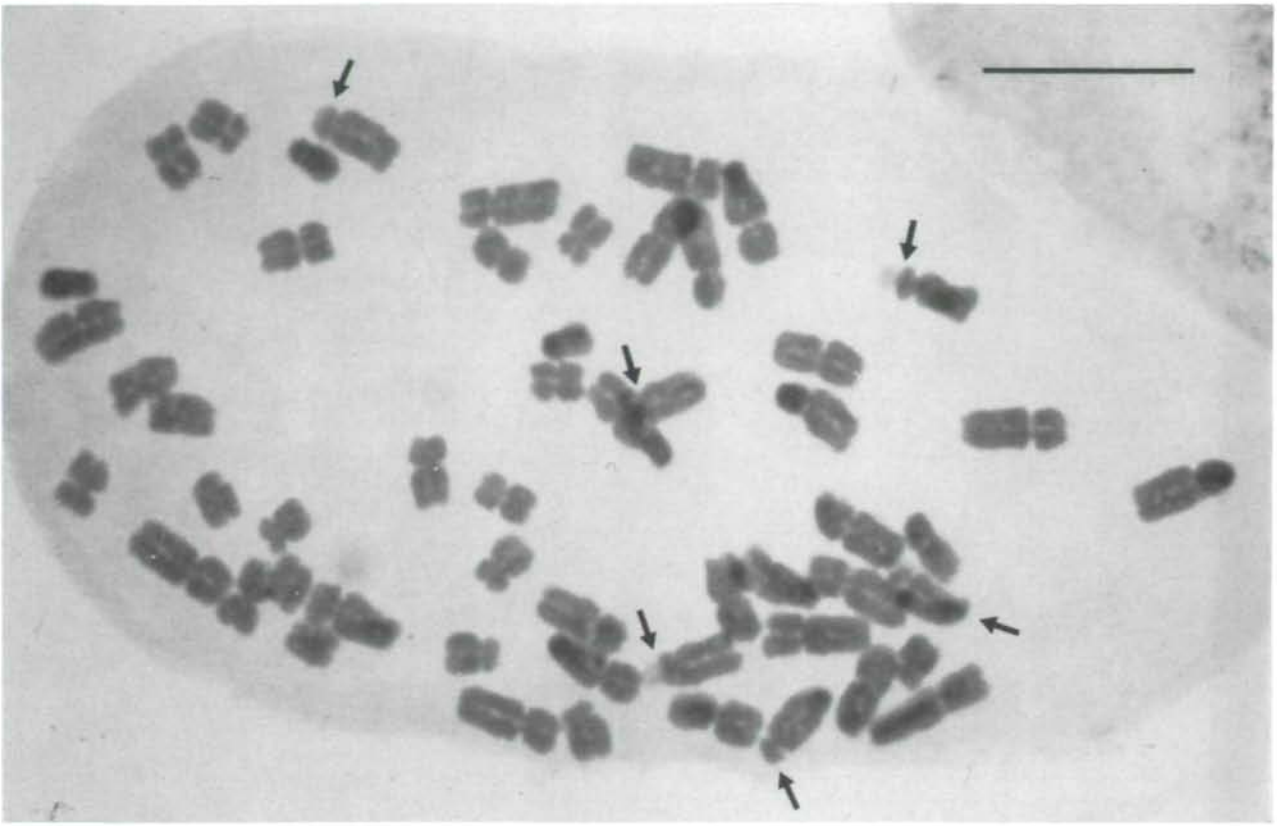

Fig. 2: Scilla bifolia agg. (2n = 54, 6x), Kreuttal. Root tip metaphase plate. Aceto - carmine. Nucleolar organizing chromosomes arrowed. Bar = 10 µm.

Another interesting taxon to be considered is S. bulgarica Speta, a provisional diploid member of Scilla sect. Luteoscilla (Speta 1980) with a surprisingly low DNA content of 1 C = 5,15 ± 0,40 pg ($\overline{q_w} \pm s_{q_w}$, 67 nuclei measured; unpublished data by J. Greilhuber). As to now this is the value most close to the value of the Kreuttal population, but is nevertheless significantly different ($t = 2{,}668 > t_{87;0,01} = 2{,}633$). It is therefore obvious, that at present statements about the phylogenetic position of the Kreuttal population would be premature. It appears that these plants represent a hitherto unique element in the Scilla bifolia aggregate and therefore possibly a new biological species. The DNA amount seems to be a sensible specific marker for this phylogenetic line, and offers the possibility for a more extensive geographical screening. It is, however, clear that in terms of the present taxonomic grouping (Speta 1980) the Kreuttal plants are a member of Scilla sect. Scilla by definition, since they have dark brown seeds, as the authors could observe.

## Acknowledgements.

As always, the cooperation of Dr. F. Speta, Oberösterreichisches Landesmuseum, Linz, is acknow-

ledged with many thanks. We are also grateful to Dr. H. Niklfeld, Institute of Botany, University of Vienna, who provided us with some data from the flore mapping project. The study was supported by "Hochschuljubiläumsstiftung der Stadt Wien".

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